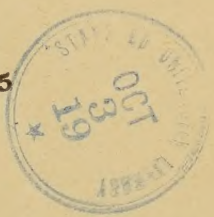
A PROXIMATE ANALYSIS OF THE ALCOHOLIC EXTRACT OF THE ROOT OF RUMEX CRISPUS, AND A COMPARISON OF THE HYDROXY-METHYL-ANTHRAQUINONES PRESENT WITH THOSE FROM CERTAIN OTHER DRUGS

BY

RUTH ELIZA OKEY
B.S. Monmouth College, 1914
M.S. University of Illinois, 1915



THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

DOCTOR OF PHILOSOPHY

IN CHEMISTRY

IN

THE GRADUATE SCHOOL

OF THE

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1918

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I wish to take this opportunity to express my appreciation of the very valuable assistance which I have received during this investigation from Dr. George D. Beal, under whose direction it was carried out.

A PROXIMATE ANALYSIS OF RUMEX CRISPUS, AND A COMPARISON OF ITS HYDROXY-METHYL-ANTHRAQUINONES WITH THOSE FROM CERTAIN OTHER DRUGS.

CONTENTS.—I. Statement of Problem. II. Historical. III. Experimental. A. Study of *Rumex crispus*. B. Investigation of Cascara. C. Investigation of Aloes. IV. Recapitulation of Results. A. Comparison of the Hydroxy methyl-anthraquinones from the Three Drugs. B. Summary and Conclusions.

I. Statement of Problem.

Rumex crispus usually known as yellow dock, is one of the commonest and most troublesome American weeds. The dried root and the fluid extract were official in the United States Pharmacopoeia of 1890, and the drug still seems to have a rather wide use in proprietary medicines.

Since there was abundant evidence in the literature that some varieties of Rumex contained hydroxy-methyl-anthraquinones, notably emodin and chrysophanic acid, and the composition of this variety was practically undetermined, the present study was undertaken in the hope that this plant might prove to be a cheaper source of these substances than cascara or aloes.

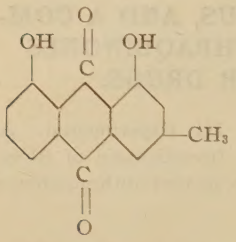
II. Historical.

Since the latter part of the eighteenth century, there have been described various naturally occurring dyestuffs which are yellow in the free state and give red colors with alkalies. The plant dyestuff preparations of the earlier workers were not, however, chemical individuals; the various "rumicins," "chrysophanic acids," etc., representing almost any combination of dye-containing materials to which the original meaning of "chrysophanic acid," *i. e.*, "I appear gold," could be applied.

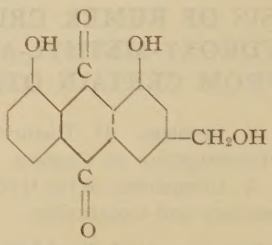
In 1858, De la Rue and Miller[1] isolated pure emodin; and in 1875 Schmidt[2] distilled aloes with zinc dust, obtaining α-methyl anthracene. This was the first real evidence of the structure of these compounds, and it remained the best evidence in the subject until 1911 when Fischer and Sapper,[3] and Fischer, Falco, and Gross[4] were able to prove that the hydrocarbon obtained from chrysophanic acid was β-methyl anthracene.

[1] *Am. J. Pharm.*, **30**, 442–447 (1858).
[2] *Ber.*, **8**, 1275 (1875).
[3] *J. prakt. Chem.*, **83**, 203 (1911).
[4] *Ibid.*, **83**, 208 (1911).

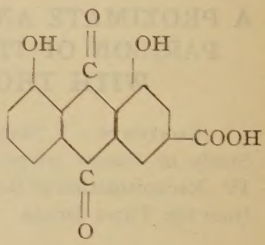
Oesterle had previously shown[1] that the substance usually associated with chrysophanic acid was emodin-monomethyl-ether, and he was also able to prove[2] that chrysophanic acid, aloe-emodin and rhein were different stages in the oxidation of the same hydroxymethyl-anthraquinone, represented by the formula:



Chrysophanic acid.

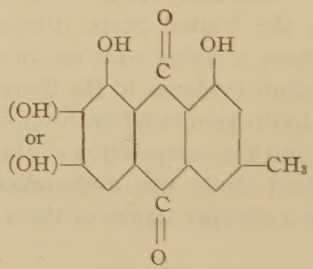


Aloe-emodin.



Rhein.

while emodin is a trihydroxy-methyl-anthraquinone, the structure of which is probably represented by the formula:



Only two modern analyses dealing with the dyestuffs of the *Rumex* family exist, and after comparing the results of these with the literature on the other plants containing hydroxy-methyl-anthraquinones it seems probable that one of them (Hesse's analysis of *Rumex nepalensis*[3]) resulted in the isolation of mixtures, rather than single dyestuffs.

The most valuable work on a plant of the *Rumex* family is that of Tutin and Clewer,[4] who isolated from the overground portion of the South African variety, *Rumex ecklonianus*, ceryl alcohol, a phytosterol, various fatty acids, ipuranol, kaempferol, emodin, chrysophanic acid, emodin-monomethyl-ether, and a sugar which yielded *d*-phenyl-glucosazone.

III. Experimental.

A. A Study of Rumex Crispus.

Material.—The material used for the analysis was obtained in part from the Meyer Brothers Drug Company of St. Louis, and from Fuller,

[1] *Arch. Pharm.*, **243**, 434 (1905); **248**, 476 (1910).

[2] *Schweiz. Wochschr.*, No. 50, (1903); *Arch. Pharm.*, **249**, 445 (1911); **250**, 301 (1912).

[3] *Ann.*, **29**, 305 (1896).

[4] *J. Chem. Soc.*, **97**, 1 (1910).

Morrison and Company, of Chicago, and collected in part by the authors in Warren and Champaign counties, Illinois. The total amount of the crude drug used was about 75 pounds, but 35 pounds was the largest amount worked with at any one time.

Preliminary Examination.—50 g. of the dried and powdered root was exhausted in a Soxhlet apparatus with the following succession of solvents:

No.	Solvent.	% dry weight of drug extracted.
1	Petroleum ether	0.536
2	Ethyl ether	0.424
3	Chloroform	0.538
4	Ethyl acetate	0.716
5	Ethyl alcohol	Over 3%

All of these extracts gave deep red colors with alkalies, indicating the presence of hydroxy-methylanthraquinones, either in the free state or as glucosides.

Systematic Analysis.—The dried root in No. 20 to No. 30 powder was exhausted by percolation with cold 95% alcohol, this extract concentrated under diminished pressure, and the concentrate precipitated by pouring it into a large excess of water, thus separating it into a water-soluble and a water-insoluble fraction.

The Water-soluble Extract was examined in three different ways: (*a*) A first portion was shaken with a large amount of ether, the ether solution separated, and the material remaining in the aqueous layer precipitated successively with neutral and basic lead acetates. After the removal of the lead as lead sulfide much resinous material was obtained, but nothing crystalline could be separated from either precipitate. The concentrated lead-free filtrate from these precipitates gave a positive reaction with Molisch's reagent, but no osazone.

The ether extract of the water solution on shaking with alkalies of various strengths, followed by acidification of these extracts, yielded in the sodium carbonate-soluble fraction a small quantity of emodin.

(*b*) A second portion of the water-soluble extract was treated with hydrochloric acid, at first only to acid reaction, when considerable turbidity resulted. The addition of more acid, up to 5% actual hydrochloric acid, was found to increase the amount of precipitate formed. The precipitate obtained with this concentration of acid was filtered off, when it was found that more precipitate formed on standing. These precipitates were united and examined together with similar precipitates obtained as described later by the action of sulfuric acid. The total weight of the dried precipitate from 10 pounds of the drug was about one gram. The second precipitation, at least, must have been caused by the hydrolysis of some water-soluble glucosidic material.

The acid filtrate was shaken successively with petroleum ether, ethyl ether and amyl alcohol. The petroleum and ethyl ether extracts yielded

residues too small in amount for further investigation, and the amyl alcohol extract yielded several different pigment fractions which were examined very carefully, but since the results were not entirely definite, the description of the process is omitted.

The acid aqueous liquid was neutralized with sodium hydroxide, and a precipitate consisting of iron, aluminum and sodium salts of various organic acids, mixed with humin material and some occluded chloride, was obtained. On clarification of the resulting filtrate no sugars could be demonstrated to be present, so it was evident that the prolonged action of the acid had resulted in the decomposition of many substances present in the original extract.

(*c*) Precipitation with sulfuric acid, followed by immediate removal of the acid with barium hydroxide, was therefore attempted. Approximately two liters of the water-soluble fraction, representing about 400 g. of the concentrated alcoholic extract of the drug, was treated with sulfuric acid to a concentration of 1% actual H_2SO_4, and allowed to stand for a few hours. Then the solution was boiled, filtered and the filtrate neutralized at once with hot saturated barium hydroxide solution. The filtrate from the precipitate barium sulfate was then clarified with alumina cream, and subjected to the usual tests for sugars.

About 10 cc. of this filtrate with 2 g. of phenylhydrazine hydrochloride and 3 g. of crystallized sodium acetate gave, after 5 minutes' heating, a copious precipitate of a crystalline osazone. After recrystallization from hot 50% alcohol, this yielded the characteristic needles of glucosazone, melting with decomposition at 205–208°.

The remainder of the clarified sugar solution was made up to 500 cc. and the rotation measured at 20° and at 25°. The resulting readings were too nearly alike to have any special significance, so a determination of the copper-reducing power of the solution was made by the method of Defren. 5 cc. portions of the solution, after dilution to 25 cc., gave 0.2816 and 0.2821 g. of cupric oxide, respectively. Calculated as dextrose, this would be equal to about 25.2 mg. of sugar in one cc., or a 2.5% solution. Substituting this value in the formula $(\alpha) = \frac{100a}{l \times c}$ where a = the observed rotation, l = the length of the tube, and c = the concentration of the solution; with the values obtained above, we have:

$$(\alpha) = \frac{100 \times 2.5 \times 0.3468}{2.52 \times 20} = 17.34.$$

Since fructose is equal in reducing power to only 91.5% of its weight of glucose, any fructose present would increase the value of c and so decrease the value of (α), aside from the fact that fructose is levo-rotatory,

and has a negative rotation greater in degree than the positive rotation of glucose. Since the dextro-rotation of glucose is greater than that observed, this sugar apparently consists of a mixture of glucose with fructose, the glucose being present in the larger proportion. An attempt to prepare a methyl-phenyl-levulosazone failed, probably because of the other sugars present.

Sulfuric acid was added to the water-soluble fraction of the remaining drug extract until the concentration was equal to 2% actual sulfuric acid. The filtrate was neutralized with barium hydroxide and treated as before, but nothing new was isolated.

After being dried, the precipitates were united with the similar ones described above, and extracted in a Soxhlet apparatus with the usual succession of solvents. The petroleum ether extract yielded almost no residue. The ethyl ether extract was shaken successively with 8% ammonium carbonate, 2% sodium carbonate and 2% sodium hydroxide solutions. From the ammonium carbonate extract, after acidification, only a small amount of an amorphous red resin was obtained, while the sodium carbonate yielded about 0.5 g. of emodin which crystallized in characteristic needles from glacial acetic acid, and was in every respect identical with that described later. The sodium hydroxide extract yielded about 0.1 g. of a substance, which, on crystallization from hot alcohol, formed the golden yellow plates characteristic of a mixture of chrysophanic acid and emodin-monomethyl-ether. From the chloroform and alcohol extracts of the acid precipitate only resinous substances were obtained.

The Water-insoluble Extract. (*a*) *Preliminary Experiments: Steam Distillation.*—A 50-g. portion of the resin was mixed with a small quantity of water and distilled with steam, the distillation being continued during the working hours of the day for 4 weeks. The distillates were shaken out with ether and the solvent removed. Although the total residues from the first fractions amounted to only a few drops, the pronounced odor and oily appearance of the distillates made it very evident that an essential oil was present.

Later, increasing quantities of a yellowish, waxy solid were obtained. On shaking the ether solution of this with sodium carbonate solution a small amount of fatty acid was removed, while the sodium carbonate-insoluble, sodium hydroxide-soluble fraction yielded a small amount of an hydroxy-methyl-anthraquinone melting at 196–197°—a fact which indicated that it must have been either chrysophanic acid or emodin-mono-methyl-ether. As the total solid material did not amount to half a gram, it was impossible to investigate its nature farther.

The aqueous layer remaining in the flask after steam distillation was filtered from the insoluble resin, and the filtrate clarified with lead acetate, the lead being removed from both filtrate and precipitate with hydrogen sul-

fide. The precipitate consisted entirely of resinous material. The concentrated filtrate gave a positive reaction with Molisch's reagent, reduced Fehling's solution very readily, gave a positive test with the orcein-phosphoric acid reagent for levulose, and yielded an osazone having the crystalline form of dextrosazone and melting, after recrystallization, at 204°. The sugar must, therefore, have been a mixture of glucose and fructose. Since any sugar originally present in the alcoholic extract would surely have been in the water-soluble fraction of that extract, this sugar must have resulted from the hydrolysis of a glucoside. What the substance in combination with the sugar was, is doubtful, but it is at least possible that this may have been an hydroxy-methyl-anthraquinone.

(*b*) *Analysis*: *Water-insoluble Resin.*—The bulk of the water-insoluble resin was dried at room temperature, mixed with purified sawdust, and exhausted successively with petroleum ether, ethyl ether, and chloroform, using a Soxhlet apparatus.

The petroleum ether-soluble substances were extracted very slowly. The first fractions contained fats in large proportion, but subsequent extraction yielded clear yellow solutions, which, on standing, deposited a partially crystalline yellow solid.

Study of Precipitated Material: Chrysophanic Acid and Emodin-mono-methyl-ether.—This solid, after repeated recrystallization from alcohol, formed in beautiful, glistening, golden yellow plates, microscopic in size. It was insoluble in sodium carbonate, but soluble in sodium hydroxide solutions with a deep red color. This fact, coupled with the irregular variation of the melting point on recrystallization (between 150° and 170°), led to the belief that this was a mixture of chrysophanic acid with emodin-mono-methyl-ether.

About 0.3 g. of the substance was therefore dissolved in cold conc. sulfuric acid, and the deep red solution slowly heated to 160°, in order to effect demethylation.[1] This solution was cooled and poured into a large excess of water, cooled again, and shaken out with chloroform. Some of the color, however, failed to be extracted, indicating that a certain amount of sulfonation had taken place.

The chloroform solution was shaken with 5% sodium carbonate until no more color was removed. On acidification, this solution yielded emodin, identical with the product described later. Subsequent shaking with 2% sodium hydroxide yielded, after acidification of the solution, shaking out with chloroform, distilling off the chloroform, and recrystallizing the residue from alcohol, deep golden yellow plates melting at 185–186°, probably chrysophanic acid which was not quite pure.

An attempt was also made to use the separation described by Oesterle,[2] as

[1] *J. Chem. Soc.*, **99**, 955, 956 (1911).

[2] *Arch. Pharm.*, **243**, 434 (1905).

follows: Approximately 0.5 g. of the material was dissolved in dry benzene, one g. of anhydrous aluminum chloride added, and the mixture refluxed on the steam bath for 4 hours. The solution was reddish purple, rather than the blue described by Oesterle. On standing for several hours after cooling, however, a deep blue precipitate settled out, and the solution above it was red. The benzene was distilled off and the residue treated with very dilute hydrochloric acid. This formed, at first, an alizarin colored emulsion, probably by the formation of an intermediate aluminum salt, which, after 15 minutes on the steam bath, was decomposed, and settled out as a yellow precipitate. This was filtered off, taken up with dilute sodium hydroxide, again precipitated with hydrochloric acid, and the dyestuff shaken out with chloroform.

The sodium carbonate extract obtained from this chloroform solution was small compared to that obtained by the treatment with sulfuric acid alone, and some of the remaining color failed to be extracted by the sodium hydroxide, due probably to the formation of neutral substances by a side reaction.

The sodium hydroxide solution, after acidification, followed by shaking out with chloroform, distillation of the chloroform, and many recrystallizations of the residue from a mixture of ethyl acetate and alcohol, yielded beautiful golden yellow plates, the melting point of which could not be raised above 175°. Sublimation of this product over a sand-bath yielded orange plates, melting sharply at 189°, approximately pure methoxyl free chrysophanic acid. The yield was very poor, and the method long, tedious and unsatisfactory.

Study of Direct Extraction. Petroleum Ether Solution.—The material remaining in solution in petroleum ether after the removal of the precipitate described above was freed from the solvent and taken up with ethyl ether. Shaking this solution directly with the different alkalies proved to be impossible, because of the permanent emulsion formed, the only definitely crystalline substance isolated by this process being a small amount of emodin which was obtained from the part of the sodium carbonate solution which formed a layer below the emulsions.

Saponification. Petroleum Ether Extract.—80 g. of the residue, representing 25 pounds of the crude drug, was therefore saponified by refluxing in the usual way with alcoholic potassium hydroxide. The alcohol was distilled off, and the residue mixed with clean sand, dried carefully, and extracted in a Soxhlet apparatus with absolute ether. This resulted in the formation of a colloidal solution of the soaps in the ether, and since this was not altered by the addition of other immiscible solvents, such as petroleum ether, nor by resaponification with a greater excess of alkali, nor by extremely careful drying, the method was abandoned. The residues from the saponification were then treated directly 16 times with cold,

dry ether, and the ether extracts so obtained shaken with water to remove traces of the alkali, the ether distilled off, and the residue taken up with 95% alcohol. They proved to consist of a phytosterol, which was obtained after about 20 recrystallizations as 1.15 g. of snow-white plates, and a hydrocarbon which was insoluble in the alcohol, but so intimately mixed with the phytosterol that it interfered with its crystallization.

The melting point of the phytosterol was 132–133°, and remained unchanged when it was mixed with a similar phytosterol obtained by the authors in their investigation of cascara. Treating this substance with acetic anhydride and a few drops of sulfuric acid resulted in the production of a red color, which changed through purple to blue and finally green and brown.[1] On combustion the results first obtained indicated that the substances contained some water of crystallization, so it was dried to constant weight at 97, after which the results indicated were obtained:

Subs., 0.1465, 0.1319; CO_2, 0.4481, 0.4012; H_2O, 0.1598, 0.1418.
Calc. for $C_{20}H_{34}O$: C, 82.8; H, 11.7. Found: C, 82.4, 83.1; H, 12.2, 12.0.

The phytosterol acetate, prepared by refluxing with acetic anhydride and fused sodium acetate, melted at 122°, and the melting point was unchanged when it was mixed with the acetate of the phytosterol from cascara. This substance was therefore the phytosterol rhamnol described by Jowett.[2]

The hydrocarbon associated with the phytosterol was present in such small amounts, and so thoroughly mixed with soaps carried over mechanically that entire purification was impossible. The best sample obtained was solid at room temperature, melted below the boiling point of alcohol, was insoluble in cold, conc. sulfuric acid, sulfonated with fuming sulfuric acid, and took up a comparatively small amount of bromine. Not enough was obtained to permit of further investigation.

The *alkaline residues* remaining after the extraction of the saponified material with ether were taken up with water, acidified, extracted with ether, and the ether solutions shaken 20 times with 5% sodium carbonate, and finally twice with 2% sodium hydroxide solution.

The sodium carbonate solutions tended to emulsify very badly, so, after acidification, they were taken up with dry ether, dried over anhydrous sodium sulfate, and the fatty acids present (approximately 60 g. in 170 g. ether) were esterified by refluxing with 100 g. of a 2.5% solution of hydrochloric acid in absolute ethyl alcohol. After 12 hours on the water bath, the solution was washed with water to remove the alcohol, then with a 5% sodium carbonate solution, which removed some emodin, together with fairly large amounts of a brown resin.

After distilling the ether from the solution of the fatty acid esters

[1] "Liebermann-Burchard Reaction," *Ber.*, **18**, 1804 (1885).
[2] *Proc. Am. Pharm. Assoc.*, **52**, 288 (1904).

they were fractionated, first at atmospheric pressure, and after the boiling temperature had risen to 200°, at 20 mm. pressure. The amounts of the various fractions are listed in the tables given below.

Weighed samples of the various fractions were saponified according to the method of Koettstorfer.[1] The acids set free by hydrochloric acid were dried to constant weight at 97° to remove occluded hydrochloric acid and the neutralization equivalents and iodine values determined by standard methods, Hanus' solution being used for the iodine numbers. That part of the 220° fraction remaining after these investigations was recrystallized several times from 90% alcohol, washed with 70% and 90% alcohol, and finally recrystallized from boiling absolute alcohol. This yielded a product melting at 62° and having a neutralization equivalent of 269.5, corresponding to a mixture of palmitic and stearic acids. Arachidic acid was, therefore, absent. No material was available for further investigation of the other fractions. The results of this investigation are tabulated below.

B. p. ester.	M. p. acid.	Sap. No. Av.	Sap. equiv. Av.	Neut. equiv. Av.	Acid No. Av.	Iodine No. Av.
208–10°..... (5.28 g.)	45°	185	300–307	317	177	47.2
220°..... (8.47 g.)	42°	190	295	299	187	59.7
235°..... (4.03 g.)	43°	183.8	305	312	179.5	31
255°..... (5.35 g.) (liq.)	28°	162	346.3	352.5	159.3	46.7
265°..... (2.62 g.) (solid)	32°	127.7	439	470	119	76.9
220°.....	62°	...	...	269.5	...	..

The saturated acids present are, therefore, palmitic and stearic, the stearic being in the larger proportion. The unsaturated erucic acid $C_{22}H_{42}O_2$ (Mol. wt. 388, m. p. 33–34°, iodine number 75.15, b. p. ethyl ester above 360° at 760 mm.) probably forms the chief constituent of the 255–265° fractions, which must also contain small amounts of fatty acids of still higher molecular weights. There is also considerable evidence of the presence of unsaturated fatty acids of lower molecular weight in the fractions of lower boiling points.

The *sodium hydroxide-soluble, sodium carbonate-insoluble fraction* extracted by ether, after decomposing the saponified petroleum ether extract with acid, yielded, after acidification, re-extraction with ether, and recrystallization from boiling alcohol, about 2 g. of a substance which crystallized in beautiful, golden spangles made up of glistening plates

[1] Sherman, "Organic Analysis," 2nd edition, pp. 144–48.

aggregating in tree-like form. These crystals melted sharply at 196°, and, acetylated by the usual method, gave an acetyl derivative crystallizing in greenish yellow hexagonal plates, melting sharply at 204°.

Combustion of this substance by the ordinary method of Liebig gave results which were too low, due to its great tendency to sublime unchanged at high temperatures, even in an atmosphere of oxygen, and in the presence of white, hot copper oxide. Using the method described by Levene and Bieber[1] with a catalyst of cerium oxide asbestos, the followingresults were obtained:

Subs., 0.1011, 0.1207; CO_2, 0.2616, 0.3166; H_2O, 0.0352, 0.0400.
Calc. for $C_{15}H_{10}O_4$: C, 70.9; H, 3.9. Found: C, 70.6, 71.4; H, 3.95, 3.7.

This was considered to complete the proof that the substance was methoxyl-free chrysophanic acid, in a very pure state.

The ethyl ether extract of the original water-insoluble resin, amounting to 18 liters, was repeatedly shaken with 8% ammonium carbonate solution in large excess. All the ammonium carbonate washings, amounting to 375 liters, were acidified, again taken into ether solution, the ether distilled off, and the residues treated with warm, glacial acetic acid. By repeating this process several times, removing the dissolved resinous matter by filtration, dissolving the residues in hot alcohol, filtering hot, allowing to cool, again filtering, and treating the residue with glacial acetic acid; and following this by repeated recrystallizations from boiling glacial acetic acid, the least soluble fraction finally was made to yield a small amount of a substance crystallizing in the orange-red needles characteristic of emodin. This substance melted at 250° and was in every way identical with the substance obtained from the sodium carbonate extract. Nothing crystalline could be obtained from the fraction more soluble in glacial acetic acid.

After the ammonium carbonate washings of this ether extract had become colorless, a 5% solution of sodium carbonate was substituted, and the extraction repeated 6 times. Since the carbonate did not become colorless, 2% sodium hydroxide was then substituted, and the extraction of the anthraquinone completed in two washings. The hydroxide solution was then saturated with carbon dioxide, and the precipitated gold-colored plates filtered off, after which the filtrate was acidified, and the precipitate so obtained added to that from the acidified sodium carbonate extract.

The *sodium carbonate-soluble fraction* on recrystallization from boiling glacial acetic acid yielded about 16 g. of the beautiful, needle-shaped crystals of emodin, which, after repeated recrystallization from alcohol, melted at 253°. On combustion, by the method described for chrysophanic acid, the following results were obtained:

[1] *J. Am. Chem. Soc.*, **40**, 460–462 (1918).

Subs., 0.09, 0.1433; CO_2, 0.2204, 0.3564; H_2O, 0.0318, 0.496.
Calc. for $C_{15}H_{10}O_5$: C, 66.7; H, 3.7. Found: C, 66.8, 66.7; H, 3.9, 3.88.

An acetyl derivative prepared by refluxing with acetic anhydride and fused-sodium acetate for one hour formed beautiful lemon-yellow needles melting at 197°—the triacetyl-emodin described in the literature. A benzoyl derivative was prepared by the Schotten Baumann synthesis, sufficient benzoyl chloride being used at the end of the process to make the solution distinctly acid. The precipitated material was extracted with 50% alcohol to remove the benzoic acid and then, after recrystallization from 95% alcohol, a product was obtained melting at 224°, and corresponding in every way to the dibenzoyl derivative of emodin described in the literature. The identity of this substance with *frangula* emodin was thus established.

The *sodium hydroxide-soluble* fraction precipitated by carbon dioxide was washed free from alkali, dried, and extracted in a Soxhlet with chloroform. After recrystallization from boiling 95% alcohol, this extract deposited golden yellow plates having the properties of the mixture of chrysophanic acid and emodin-mono-methyl-ether described previously.

Neutral substances were present in the ether extract of the original water-insoluble resin in amounts too small for investigation.

A careful examination of the chloroform extract of this resin was made, but nothing crystalline could be isolated. This was likewise true of the alcohol extract.

B. Investigation of Cascara.

This was undertaken primarily in order to obtain the hydroxy-methyl-anthraquinones for purposes of comparison; and since H. A. D. Jowett[1] has published a more complete analysis of the drug, the present work will not be reported in detail. The analysis followed, with minor exceptions, the method used by the authors for *Rumex*, except that only a 10-pound sample was worked up.

The water-soluble portion yielded, in the ether solution, very small quantities of emodin, and in the filtrate from the lead acetate precipitate, a sugar which gave *d*-phenyl-glucosazone and a positive orcein-phosphoric acid test for fructose.

The water-insoluble resin yielded, in the petroleum ether extract, fairly large quantities of fatty acids soluble in sodium carbonate, from which arachidic acid was separated and definitely identified. The sodium hydroxide extract of the petroleum ether solution yielded only very small amounts of emodin, while the neutral fraction, after saponification, gave a second quantity of fatty acids and the phytosterol which was proven to be identical with that isolated from *Rumex crispus*.

The ethyl ether extract of the water-insoluble resin yielded emodin

[1] *Proc. Am. Pharm. Assoc.*, **52**, 288 (1904).

melting at 252° and forming an acetyl derivative melting at 197° which proved to be in every way identical with that from *Rumex crispus*. A careful study was made of the acetyl derivatives of emodin from the two sources, and it was found that while some changes could be made in the crystalline form and in the color by crystallizing from different solvents and at different temperatures, the melting points and the solubilities remained the same. The needles described in the literature are, however, really prisms, and the changes in the crystalline form consist in their becoming broader or narrower, according to the conditions under which they separate from the solvent.

The chloroform and ethyl alcohol extracts of the water-insoluble resin yielded no definite compounds.

C. Investigation of Aloes.

This was likewise undertaken in order to obtain a sample of aloe-emodin. Approximately 400 g. of Barbadoes aloes obtained from the Meyers Bros. Drug Company of St. Louis was ground up, mixed with a small quantity of alcohol, and exhausted by percolation with cold 95% alcohol. The residue left after removing the alcohol from this percolate was mixed with purified sawdust, dried and exhausted in a Soxhlet apparatus with petroleum and then with ethyl ether.

The residue from the petroleum ether extract was very small in amount, and yielded only fat and fatty acids, while the ethyl ether extract, likewise small, gave only resinous material in the ammonium and sodium carbonate extracts, and in the sodium hydroxide-soluble fraction a small amount of a substance which crystallized in the characteristic orange-red needles of aloe-emodin. This substance melted at 224°, and gave, when refluxed with acetic anhydride and fused sodium acetate, an acetyl derivative which melted at 175–176°—the diacetyl aloe-emodin described in the literature. A benzoyl derivative prepared by the Schotten-Baumann reaction melted at 237–238°—the tribenzoyl aloe-emodin described by Tutin and Naunton.[1] The identity of this substance as aloe-emodin was therefore considered to have been established.

Since the yield of anthraquinone derivatives had been so small, it was considered expedient to attempt hydrolysis of the resin remaining, so this was again extracted with alcohol, the solvent removed and the resin treated with a 2% aqueous solution of sulfuric acid. The mixture was placed in 4-liter flask, covered with about two inches of benzene, placed on a water bath and connected with a reflux condenser and a capillary tube through which a current of air was drawn in order to stir the mixture. As the hydrolysis proceeded and the benzene became saturated, it was replaced by fresh portions of this solvent.

These benzene extracts were freed from the solvent, the residues taken

[1] *Pharm. J.*, **91**, 836 (1913).

up with ethyl ether, and this solution shaken successively with the usual alkalies. The ammonium carbonate removed cinnamic acid, identified by its melting point, its behavior on oxidation with potassium permanganate, and the properties of the *p*-nitro derivative. From the sodium hydroxide-soluble fraction larger amounts of aloe-emodin identical with the product described above were isolated.

IV. Recapitulation of Results.

A. Comparison of the Hydroxy-methyl-anthraquinones from the Three Drugs.

The emodin of *Rumex crispus* forms orange-red needles, melting at 250–255° when crystallized from alcohol or pyridine; gives an acetyl derivative forming lemon-yellow prisms which melt at 197°, and a dibenzoyl derivative which melts at 224°. This emodin is soluble in alcohol, hot glacial acetic acid, ether, benzene, and chloroform, and in solutions of fixed alkali carbonates and hydroxides, properties which check in every way those of the emodin from cascara.

The identity of these emodins has further been proven by the fact that the melting point remains the same when the two compounds are mixed. This would indicate, moreover, that the emodin of *Rumex crispus* is also identical with that from *Rumex ecklonianus*,[1] with that from rhubarb,[2] and with that described by various writers from *Rhamnus frangula*.

It differs from the emodin of aloes, and consequently from that of senna,[3] in that this latter compound melts at 224°, forms an acetyl derivative which melts at 177° and a tribenzoyl derivative which melts at 235°. Aloe-emodin is much less soluble in cold solutions of alkali carbonates, and in cold alcohol. The chief structural difference between the two isomers is that aloe-emodin has only two hydroxyl groups in the ring and one on the side chain, while emodin has three hydroxyls in the ring and none on the side chain, which accounts for these differences in behavior.

While no chrysophanic acid has been prepared from a drug other than *Rumex* during this investigation, and since the properties of the compound from *Rumex crispus* check in every way those of the compounds isolated by Tutin from rhubarb, and from *Rumex ecklonianus*, which were described as crystallizing in golden yellow spangles melting at 197°, and yielding an acetyl derivative forming greenish yellow plates which melt at 204°, are very slightly soluble in ether and cold alcohol and more soluble in ethyl acetate and chloroform, there is very conclusive evidence that the compound from *Rumex crispus* is identical with those from these drugs.

[1] Tutin and Clewer, *J. Chem. Soc.*, **97**, 1 (1911).
[2] *Ibid.*, **99**, 946 (1912).
[3] Tutin, *Ibid.*, **103**, 2006 (1913).

The mixture of chrysophanic acid and emodin-mono-methyls ether isolated from *Rumex crispus* has properties identical with those described for a similar mixture obtained by the oxidation of chrysarobin by Oesterle and his co-workers.[1]

The combination of hydroxy-methyl-anthraquinones present in *Rumex crispus* is not the same as that described for any other family of plants, although it agrees exactly with that of *Rumex ecklonianus*. Cascara contains emodin and perhaps an iso-emodin; senna, aloe-emodin and rhein; aloes, aloe-emodin; and rhubarb, not only rhein, emodin, and emodin-mono-methyl-ether, but also chrysophanic acid, aloe-emodin, and rheinolic acid. There seems, however, to be some possibility that *Rhamnus frangula* may contain the combination of emodin, emodin-mono-methyl-ether and chrysophanic acid which is present in *Rumex*.

B. Summary and Conclusions.

(1) The following substances are present in the material extracted from the dry root of *Rumex crispus* by cold 95% alcohol:

(*a*) Soluble in water: Small amounts of emodin and a mixture of emodin-mono-methyl-ether and chrysophanic acid; a pigment which is probably related to the anthocyanins; sugars yielding *d*-phenyl-glucosazone and having properties indicating the presence of fructose and invert sugar as well as glucose, besides organic acids and much resinous material. It is very probable that some of these substances are present in the plant in the form of glucosides.

(*b*) Insoluble in water: emodin, emodin-mono-methyl-ether, chrysophanic acid, a phytosterol, palmitic, stearic and erucic acids, together with unsaturated fatty acids of lower and saturated fatty acids of higher molecular weights, a small amount of an unidentified hydrocarbon, probably a terpene, an essential oil, and a large percentage of resinous material. The presence of glucosides is clearly indicated.

(2) The emodin isolated from *Rumex crispus* is identical with that from cascara (*Rhamnus purshina*) and the phytosterol has likewise been proven to be identical with the rhamnol from that plant.

(3) The yield of emodin from the dried root of *Rumex crispus* amounted to about 0.1% of its weight, and that of chrysophanic acid was somewhat less. This compares favorably with the yields from more expensive drugs, and it is very probable that methods can be worked out which will very considerably increase this yield.

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[1] *Arch. Pharm.*, **246**, 476 (1910); *Ibid.*, **249**, 445 (1911).

VITA.

The writer was born near Woodsfield, Ohio, in 1893. She attended the public schools of that town, graduating from the High School in 1911. She entered Ohio University in the fall of that year, and remained there one year. She matriculated at Monmouth College in 1912, from which institution she was graduated with the Degree of Bachelor of Science in Chemistry in 1914, receiving the nomination of the college faculty for a scholarship in the Graduate School of the University of Illinois for the year 1914–1915. She completed the work for the degree of Master of Science in Chemistry at the University of Illinois in 1915, and taught there as a Graduate Assistant in Chemistry during the years 1915–1917. In 1917 she was granted a fellowship in Chemistry at the University of Illinois. She is a member of Iota Sigma Pi and Sigma Xi. With Dr. George D. Beal she has published "The Qualitative Identification of Drugs Containing Emodin." [1]

[1] *J. Am. Chem. Soc.*, **39**, 716–725.